REMARKS

Claims 1-15 were originally presented. New claims 16-19 were presented in the response filed December 17, 2007. Claims 1-8, and 18 were revised and new claims 20-21 were submitted in the response filed August 15, 2008. Claims 6, 8, 16, 18 and 19 were amended in the supplemental amendment to Request for Continued Exam, filed on November 5, 2008. Claims 1 and 2 are amended herein to better define the invention. The claims presently under consideration are thus claims 1-21, as set forth herein. These claims are supported by the specification as filed, and Applicant believes that no new matter has been added. Applicant respectfully requests that the Examiner reconsider and withdraw the various grounds of rejection of the claims.

Detailed Action

On page two of the Office Action, the Examiner has acknowledged Applicant's request for continued examination filed on October 16, 2008. The Examiner also acknowledges the information disclosure statement filed on May 30, 2008 and the declaration filed on August 15, 2008.

On page three of the Office Action the Examiner withdraws all rejections set forth in the prior action mailed April 16, 2008 but for the rejection of claims under 35 USC 112 2nd paragraph.

35 USC 112

The Examiner rejects claims 1-21 under 35 USC 112 second paragraph, as being indefinite for failing to particularly point out and distinctly clam the subject matter which applicant regards as the invention. Particularly the Examiner objects to the limitation "wherein said diffusion provides extended or time-release delivery of said ophthalmic drug". The Examiner states that such limitation is indefinite because diffusion does not provide release characteristics; the materials utilized do.

Applicant traverses this rejection. Without acquiescing to this rejection and solely to expedite allowance of pending claims, Applicant has amended claim 1 to read as follows: "wherein said ophthalmic drug is capable of diffusion into and migration through said contact lens and into the post-lens tear film when said contact lens is placed on the eye; wherein said diffusion results in extended or time-release delivery of said ophthalmic drug". Applicant submits the rejection is rendered moot by this amendment. Should the Examiner believe otherwise, the Applicant requests a teleconference to better understand this rejection and to discuss with the Examiner potential alternative language.

35 USC 103

Claims 1-5, 7-15 and 20-21 are rejected by the Examiner as being unpatentable over Resnick (US 2002/0141760) (hereinafter "Resnick"); Ding (PSTT, Vol. 1, No. 8, Nov 1998) (hereinafter "Ding"); Vandamme (Progress in Retinal and Eye Research 21 (2002) 15-34) (hereinafter "Vandamme"); Nagarsenker et al (Int. Journal of Pharmaceutics 190 (1999) 63-71) (hereinafter "Nagarsenker"); and Paul et al (Current Science, Vol. 80, No8, 25 April 2001) (hereinafter "Paul"). Claims 6 and 17-19 are rejected as being unpatentable over Resnick; Ding; Vandamme; Nagarsenker; Paul and Darouger et al (US 6,264,971) (hereinafter "Darouger ").

First, it is noted that the Examiner requires no less than five (5) separate references to reject the claims 1-5, 7-15 and 20-21 and no less than six (6) separate references to reject the claims 6 and 17-19. Moreover, the Examiner's rejection is "piecemeal"; i.e., to establish a *prima facie* case of obviousness, the Examiner is obliging the skilled artisan to piece disparate elements of the present invention from each of the multiple cited references.

Applicant is aware that the requirement of a large number of references and a specific arrangement thereof to meet all the limitations of a claim do not necessarily invalidate the grounds of rejection. However, the fact that such a unique, multi-tiered combination of five and six references is required herein certainly complicates the Examiner's burden of reasonably connecting so many disparate teachings in a logical and coherent manner in order to establish a *prima facie* case of obviousness. See Ex *parte Gambogi*, 62 USPQ2d 1209; *In re Herrick*, 145 USPQ 400; *In re Gorman*, 18 USPQ2d 1885; *In re Yates*, 211 USPQ 1149; *In re Rijckaert*, 28 USPQ2d 1955; *Corometrics v, Berkeley*, 193 USPQ 467; and *Gen. Tire v. Firestone*, 174 USPQ 427. It will be apparent from the following discussion that the Examiner has not shouldered that burden and that the claimed invention is patentable.

Applicant continues to submit that the focus and intent of Resnick's invention is very different from that of the current invention, and that Resnick does not enable a person of ordinary skill to load nano/microspheres into contact lenses for the purpose of extended drug delivery.

Applicant further notes that Resnick published on **October 3, 2002**. The present application is a Continuation in Part of 10/454,836 filed 6/5/2003 (now issued patent 7,638,137), and claims priority to provisional application 60/385,571 filed **June 5, 2002**.

Applicant therefore submits that given Applicant's priority date **prior** to Resnick's publication date – Resnick is not a proper prior art reference to the present application.

To be considered as a reference under 35 USC 103(a) the reference must be prior art within 35 USC 102. Applicant provides 35 USC 102 (a), (b), and (e) below:

> A person shall be entitled to a patent unless -
>
> (a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, **before the invention thereof** by the applicant for patent, or
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, **more than one year prior to the date of the application for patent in the United States**, or
>
> (e) the invention was described in - (1) an application for patent, published under section 122(b), by another filed in the United States **before the invention by the applicant** for patent or (2) a patent granted on an application for patent by another filed in the United States before the invention by the applicant for patent, except that an international application filed under the treaty defined in section 351(a) shall have the effects for the purposes of this subsection of an application filed in the United States only if the international application designated the United States and was published under Article 21(2) of such treaty in the English language; or
>
> (emphasis added)

Applicant notes that given that the priority date of Applicant's invention is prior to the publication of Resnick, a declaration under 37 C.F.R. § 1.131 should not be needed. If the Examiner believes otherwise, the Applicant requests further clarification.

Ding relates to ophthalmic drug delivery by way of eye-drops. Ding provides absolutely no mention or consideration as to implementing a delivery system using a contact lens as a vehicle.

Applicant notes that Vandamme lists a publication date of 2002. The month of publication is not apparent from the article. The present application is a Continuation in Part of 10/454,836 filed 6/5/2003 (now issued patent in 7,638,137), and claims priority to provisional application 60/385,571 filed June 5, 2002. It is therefore highly likely that

Vandamme does not qualify as prior art to be cited against the present invention. The Applicant requests the Examiner to confirm the month of Vandamme publication within 2002 so that Applicant can determine whether he must swear behind the reference pursuant to 37 C.F.R. § 1.131. Regardless, Applicant notes that Vandamme provides absolutely no mention or consideration as to implementing a delivery system using a contact lens as a vehicle. The studies in Vandamme related to administration via eye drops.

Nagarsenker discloses ophthalmic drug administration via drops and/or gels. Again, there is absolutely no mention or consideration as to implementing a delivery system using a contact lens as a vehicle

Paul et al provide an overview of potential applications of compartmentalized systems of microemulsions. The Examiner refers to page 995 of Paul as teaching that "micro-emulsions allow sustained release or controlled drug release for ocular administration" Applicant submits a delivery system such as contemplated by Paul is fundamentally different from dispersions of the nanoparticles in contact lenses. Paul states: " Microemulsions are promising delivery systems [...] to allow sustained or controlled drug release for percutaneous, peroral, topical, transdermal, ocular, and parenteral administration." Paul provides absolutely no mention or consideration as to implementing a delivery system using a contact lens as a vehicle.

Darougar's patent teaches ophthalmic drug delivery through a device that is cylindrical in shape with a length of at least 8 mm and a maximum diameter not exceeding 1.9 mm. This device is designed to be inserted into the upper or the lower fornix. The teachings of this patent are substantially different from the present claims in view of the significant differences in the shape and the site of insertion. Contact lenses cover the cornea and thus are preferably transparent. Also, the contact lenses have to be kept hydrated in the packaging solution so that these do not change shape after insertion in the eye. Further, the contact lenses are only about 100 micron thick. The geometry of the device plays a very important role in drug delivery rates to the cornea.

Thus, Applicant notes that all the cited references (except Resnick) focus on ophthalmic drug delivery through ocular formulations that contain nanoparticles such as microemulsions or liposomes dispersed in aqueous solutions. These systems are fundamentally different from dispersions of the nanoparticles in the contact lenses because of the following reasons:

(1) Nanoparticles can become destabilized during polymerization. In this case, nanoparticles will not impact drug release from the gels.

(2) The resistance offered by nanoparticles to drug release may be less than that by lenses. In this case, nanoparticles will not impact drug release from the gels.

(3) The nanoparticles can release much of the drug during packaging. In this case too, the packaged lenses will have a burst release with no long term delivery.

(4) The nanoparticles can aggregate during polymerization leading to loss of transparency.

Issues one through three above are further discussed in the article provided as Appendix A. The data provided in this reference clearly shows that just putting any nanoparticles in the lenses simply does not work. The issues listed above are clearly not trivial to either understand or implement, and thus a person with ordinary skills cannot design the nanoparticle-laden lenses without undue experimentation.

Applicant has earnestly endeavored to place the application in condition for allowance and favorable action toward that end is respectfully requested. The Commissioner is hereby authorized to charge to Deposit Account No. 50-1165 (T2315-908542US02) any fees under 37 C.F.R. §§ 1.16 and 1.17 that may be required by this paper and to credit any overpayment to that Account. If any extension of time is required in connection with the filing of this paper and has not been separately requested, such extension is hereby requested.

Respectfully submitted,

Date: February 25, 2010

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Appendix A

The article *Timolol transport from microemulsions trapped in HEMA gels* follows this page.

Available online at www.sciencedirect.com
ScienceDirect
Journal of Colloid and Interface Science 315 (2007) 297–306
JOURNAL OF Colloid and Interface Science
www.elsevier.com/locate/jcis

Timolol transport from microemulsions trapped in HEMA gels

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Received 8 March 2007; accepted 11 June 2007
Available online 30 June 2007

Abstract

Approximately 90% of all ophthalmic drug formulations are now applied as eye-drops. While eye-drops are convenient and well accepted by patients, about 95% of the drug contained in the drops is lost due to absorption through the conjunctiva or through the tear drainage. A major fraction of the drug eventually enters the blood stream and may cause side effects [J.C. Lang, Adv. Drug Delivery Rev. 16 (1995) 39–43; C. Bourlais, L. Acar, H. Zia, P.A. Sado, T. Needham, R. Leverge, Prog. Retinal Eye Res. 17 (1998) 33–58; M.P. Segal, FDA Consumer Mag. (1991)]. The drug loss and the side effects can be minimized by using microemulsion-laden soft contact lenses for ophthalmic drug delivery [D. Gulsen, A. Chauhan, Invest. Ophthalmol. Vis. Sci. 45 (2004) 2342–2347; D. Gulsen, A. Chauhan, Abstr. Pap. Am. Chem. Soc. 227 (2004) U875]. In order for microemulsion-laden gels to be effective, these should load sufficient quantities of drug and should release it a controlled manner. The presence of a tightly packed surfactant at the oil–water interface of microemulsions may provide barrier to drug transport, and this could be used to control the drug delivery rates. In this paper we focus on trapping ethyl butyrate in water microemulsions stabilized by Pluronic F127 surfactant in 2-hydroxyethyl methacrylate (HEMA) gels and measuring the transport rates of timolol, which is a beta-blocker drug that is used for treating a variety of diseases including glaucoma. The results described here show that microemulsion-laden gels could have high drug loadings, particularly for drugs such as timolol base which can either be dissolved in the oil phase or form the oil phase of the microemulsions. However, the surfactant covered interface of the Pluronic microemulsions does not provide sufficient barrier to impede the transport of timolol, perhaps due to the small size of this drug.

Keywords: Hydrogel; Microemulsions; Transport; Timolol; Pluronic

1. Introduction

Topical delivery via eye-drops, which accounts for about 90% of all ophthalmic formulations, is extremely inefficient, and in certain instances leads to serious side effects [1–3]. Only about 5% of the drug applied as drops penetrates the corneal epithelium and reaches the ocular tissue while the rest is lost to systemic circulation through various pathways [1,4]. In some instances, the systemic uptake of drug can lead to undesirable side effects. For example, beta-blockers such as timolol that treat wide-angle glaucoma have a deleterious effect on the heart [5]. In view of the limitations of ocular drug delivery by eye drops, a significant amount of research has been conducted at developing more effective ophthalmic drug delivery systems based on polymeric gels such as hyaluronic acid (HA) [6], poly(acrylic acid) (PAA) [7,8], cellulose [9], soaked collagen shields [2], biodegradable and non-erodible implants [2], and colloidal particles such as liposomes [10,11], nanocapsules [12–15], contact lenses [16,17], and microemulsions.

Microemulsions are dispersions of oil in water or water in oil that are thermodynamically stable due to the significant lowering of the interfacial tension by the adsorption of amphiphiles on the surface. They have received considerable attention due to numerous applications in a wide variety of areas such as separations, reactions, drug delivery, and detoxification [18,19]. In all the applications listed above, the process of mass transfer across the surfactant-covered interface plays a key role. Most studies on ophthalmic use of microemulsions focused on entrapping hydrophobic drugs in the oil phase. Delivering ophthalmic drugs via microemulsions leads to an increase in bioavailability but the effect is rather limited because the microemulsions get washed away by tear circulation. The elimination of microemulsions with tear circulation can be avoided if these are

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0021-9797/$ – see front matter
doi:10.1016/j.jcis.2007.06.054

trapped in soft contact lenses. When a drug-laden contact lens is placed on the eye, the drug from the lens will diffuse into a thin fluid layer trapped between the lens and the cornea, namely the post-lens tear film (POLTF). There is limited mixing between the fluid in the POLTF and the outside tear fluid [20,21]. Thus, the drug released from the lens will have a long residence time in the eye. A few clinical studies have shown that the soaked contact lenses can achieve desired therapeutic results [22–33]. It has been shown by both modeling and experiments that when ophthalmic drugs are delivered as drops, only about 1–5% of the drug enters the cornea [1,4]. It has been shown by a general mathematical model that the bioavailability, which is the fraction of the applied drug that enters the cornea could be as high as 50% for drugs delivered by contact lenses [34].

Glaucoma is an ocular disease typically characterized by abnormally high intraocular fluid pressure, which can lead to partial to complete loss of vision. Approximately three million Americans have glaucoma, and as many as 120,000 are blind from this disease. Timolol, a nonselective beta-blocker, treats glaucoma by lowering the pressure inside the eye by inhibiting the production of aqueous humor. It is known that systemic absorption of beta-blockers such as timolol causes a number of side effects, such as cardiac arrhythmias and severe bronchospasms, syncopes, cerebrovascular events, heart failure, depression, states of confusion, and impotence. These side effects can be caused simply by its ophthalmic use to treat glaucoma, and can be avoided by using a more efficient ophthalmic drug delivery system. In view of the toxic side effects of timolol, a number of researchers have studied the uptake of timolol in gels composed of mixtures of various monomers that include hydroxyethyl methacrylate (HEMA), methacrylic acid (MAA), *N*,*N*-diethylacrylamide (DEAA), 1-(tristrimethyl-siloxysilylpropyl)-methacrylate (SiMA), *N*,*N*-dimethylacrylamide (DMAA), methyl methacrylate (MMA) [35–40]. Also several researchers have developed microemulsions laden with timolol for improved ophthalmic drug delivery [41]. This paper combines the microemulsion based therapies with the approach of using contact lenses by developing poly(hydroxyethyl methacrylate) (PHEMA) gels that contain dispersions of timolol-laden microemulsions. It is noted that in addition to being used for treating glaucoma, timolol is also commonly used for other therapies, and so several researchers have investigated transdermal delivery of timolol by numerous approaches including using microemulsions, patches and by iontophoresis [42,43]. While our primary motivation is development of timolol loaded gels for glaucoma therapy, the results reported here could also be useful in delivery of timolol through skin.

2. Materials and methods

2.1. Materials

HEMA monomer and ethylene glycol dimethacrylate (EGDMA) were purchased from Aldrich Chemicals (St Louis, MO); ethyl butyrate and benzoyl peroxide (BP) (97%) were purchased from Aldrich Chemicals (Milwaukee, WI); timolol maleate, Pluronic F127, Dulbecco's phosphate buffered saline (PBS), sodium caprylate, and sodium hydroxide pellets (99.998%) were purchased from Sigma Chemicals (St Louis, MO); Darocur TPO was kindly provided by Ciba (Tarrytown, NY).

2.2. Methods and procedures

2.2.1. Preparation of Pluronic microemulsions

The microemulsions described below comprise of ethyl butyrate (oil), Pluronic F127 (surfactant) and sodium caprylate (co-surfactant). The drug (timolol) needs to be loaded into the oil phase of the microemulsion but timolol maleate is a water soluble salt, and so it is not suitable for entrapment in the microemulsion. It was thus decided to convert the maleate salt to the base form of timolol, which is an oily liquid at room temperature, and then load it in the oil drops of the microemulsions. The fraction of timolol base in the oil phase and also the fraction of the oil phase in the microemulsions are varied to develop systems with different drug loadings. Four types of microemulsions are described below. They are referred to as meA, meB, meC, and meD.

The oil phase of these microemulsions is a mixture of the base form of the drug and ethyl butyrate. The timolol base was prepared by dissolving timolol maleate salt in a high pH solution, and then extracting the oily timolol base with ethyl butyrate. Specifically, 0.0831 g of timolol maleate salt was added to 6 ml of 0.77 M NaOH solution. The pH of the resulting solution is above the pK_a of timolol ($pK_a \sim 9.2$), and thus a majority of the drug gets converted to the base form, which is an oily liquid at room temperature. The mixture was then allowed to phase separate into an aqueous phase and the oily timolol phase. After phase separation, 5 ml of the aqueous phase was removed, and then 400 μl of ethyl butyrate was added to the remaining mixture. The mixture was stirred and then allowed to phase separate into an ethyl butyrate rich phase that contained a majority of the timolol base and an aqueous phase. After phase separation, the oil phase (timolol base dissolved in ethyl butyrate) was carefully pipetted out. This solution of drug and ethyl butyrate (referred as T/E) was utilized as the oil phase while preparing the microemulsions.

MeA is oil-in-water microemulsion stabilized by Pluronic F127 surfactant and sodium caprylate co-surfactant. To make the surfactant solution, 1.2 g of Pluronic F127 and 0.0163 g of sodium caprylate were added to 9 ml saline (0.85 wt% NaCl in DI water), and then stirred at 600 rpm at room temperature for a period of 5 h. Next, 0.1 ml T/E and 0.5 ml of 1.5 M NaOH solution were added to 4.5 ml of the surfactant solution with stirring at 600 rpm. After about 3 h, the solution turned clear, which indicated microemulsion formation.

MeB was prepared by the following the same procedures as for meA, except that 0.15 ml of T/E was added to 4.5 ml of surfactant solution instead of 0.1 ml.

In meC, pure timolol base was used as the oil phase instead of a mixture of timolol and ethyl butyrate. To prepare meC, 0.1642 g of timolol maleate was added to 6 ml of 1.5 M NaOH solution, and the mixture was allowed to phase separate. Next, 5 ml of the aqueous phase was pipetted out, and the remain-

Table 1
Compositions of various Pluronic microemulsions (me)

ID	Oil% in me	Surf% in me	Drug% in oil	Drug% in me
A	1.73	10.4	14.76	0.254
B	2.48	10.3	14.6	0.362
C	2.18	15.5	100	2.18
D	1.96	13.4	30	0.58

Table 2
Physical and transport properties of drugs of interest

	Timolol
Molecular weight	316
Diffusivity in tears $\times 10^{12}$ (m^2/s)	500
Dosage (drops/day)	2 (0.25%)
Corneal permeability (m/s)	1.5×10^{-7}
Fraction that enters eye	1.3%
Therapeutic requirement (μg/day)	1.5

ing mixture was dried by blowing nitrogen for about 30 min. Separately, 2.145 g of Pluronic F127 and 0.016 g of sodium caprylate were dissolved in 8 ml saline (0.85 wt% NaCl in DI water) for use as the surfactant solution for meC. Next, 1 ml of 2.31 M NaOH solution, 4 ml surfactant solution, and 0.383 g more Pluronic F127 were added to the timolol base with stirring at 600 rpm at room temperature for 3 h.

MeD was also made by similar procedures as meA, except that it had a slightly higher content of timolol in T/E mixture, a slightly higher oil content in the microemulsion, as well as a higher total amount of surfactant added to the microemulsion, while keeping the ratio of surfactant to oil constant. In addition, there was no co-surfactant added. Specifically, 0.1222 g of timolol maleate was added to 6 ml of 0.77 M NaOH solution to generate timolol base, and the mixture was allowed to phase separate. Next, 5 ml of the aqueous phase was pipetted out, and 230 μl of ethyl butyrate was added to the remaining mixture. The drug loaded ethyl butyrate was then carefully withdrawn from the mixture. Separately, 1.64 g of Pluronic F127 was dissolved in 9 ml saline (0.85 wt% NaCl in DI water) as the surfactant solution for meD. We added 0.1 ml of the drug containing ethyl butyrate and 0.5 ml of 1.5 M NaOH solution to 4.5 ml of the surfactant solution, and stirred the above solution at 600 rpm at room temperature for 3 h.

The compositions of the four types of microemulsions described above are summarized in Table 1. Also the relevant physical and transport properties of timolol are presented in Table 2.

2.2.2. Entrapment of Pluronic microemulsions in HEMA gels

The microemulsion-loaded p-HEMA hydrogels were synthesized by free radical solution polymerization with UV initiation. Specifically, 1.35 ml of the monomer (HEMA), 5 μl of crosslinker (EGDMA), and 1 ml of the microemulsion were mixed together in a glass tube. This solution was degassed by bubbling nitrogen for 15 min to reduce the amount of dissolved oxygen, which can be a scavenger of both initiating and propagating species in free radical polymerization. Next, 3 mg of the photoinitiator, Darocur TPO, was added to the mixture, and the solution was stirred for 10 min. The resulting mixture was poured in between two glass plates separated by a 200 μm plastic spacer. The mold was then put on a UVB-light illuminator for 40 min for gel curing. Control (without microemulsion) HEMA gels were prepared by following the same procedure described above, except that the microemulsion was replaced by an equal volume of DI water. The base form of timolol was loaded into the control gels by directly dissolving the drug in the polymerizing mixture.

2.2.3. Preparation of polymerizable Pluronic microemulsions with HEMA–water as the continuous phase

The microemulsions described above (meA, B, C and D) could potentially get destabilized after addition of the HEMA monomer. To eliminate this possibility, it was decided to develop microemulsions that already contain HEMA in the continuous phase, and so these can be polymerized without significant changes to the composition. Since the hydrogels are prepared with 40% water, the continuous phase of these microemulsions needs to contain HEMA and water in 60:40 ratio. The solubility of ethyl butyrate considerably increases to about 10% w/w on with addition of 60% HEMA (w/w) to water, and so microemulsions can only form at an oil loading of larger than 10%. High oil content may impede the process of gel formation, and so it was decided to increase the ionic strength of the continuous phase, which is expected to result in a reduction in the solubility of oil. Several experiments were conducted to determine the effect of salt (NaCl) and sodium hydroxide (NaOH) concentration on solubility of ethyl butyrate, and based on these experiments the composition of the continuous phase was eventually fixed to be HEMA/H_2O/NaCl/2 N NaOH solution = 53.6:35.8:1.6:9 (w/w). The fractions of oil (ethyl butyrate) and the surfactants (Pluronic F127) were then varied to investigate the compositions at which microemulsions form. The phase behavior of these six component systems was investigated at two different temperatures. These microemulsions were polymerized by adding 0.12% Darocur TPO and 0.2% EGDMA to the microemulsion, and then pouring the mixture between two glass plates separated by a 200 μm plastic spacer, and finally exposing the mold to UVB-light for 40 min for gel curing.

2.2.4. Particle size measurements

The particle size distributions of the microemulsions were measured with a Precision Detectors PDDLS/CoolBatch+90T instrument. The data were analyzed with the Precision Deconvolve32 Program. The measurements were taken at 20 °C and 90° scattering angle, using a 683 nm laser source.

2.2.5. Drug release experiments for microemulsion-laden gels
2.2.5.1. Timolol release in DI water with water replacement every 24 h After polymerization, each gel was removed from the glass mold, and was cut into pieces that were about 1.5 cm in length and width and about 200 μm in thickness. Each piece

Table 3
Fractional drug release in the extraction step for various types of microemulsion-laden gels

Gel type	Gel weight (g)	Initial timolol input (mg)	Released during initial soaking	
			(mg)	(%)
A	0.060	0.095	0.016	17.5
B	0.064	0.144	0.018	12.55
C	0.072	0.848	0.064	7.56
D	0.049	0.179	0.023	12.67

of gel was dried in air overnight and then weighed the next day. The gel was then submerged in 200 ml deionized (DI) water bath under minimal stirring and at room temperature for 5 h to extract the unreacted monomer. This step is referred to as the extraction or the initial soaking step. The extraction step was typically conducted at room temperature, but in some instances a higher temperature was used to investigate the effect of temperature on drug release rates. The fraction of drug that diffused out during the extraction step was determined by measuring the absorbance at wavelengths near the absorbance peak of timolol (295 nm). The fractions of drug released in the room temperature extraction for various types of gels are listed in Table 3.

After the extraction step, the gels were transferred into 3 ml of DI water for the drug release experiments. The DI water was replaced every day, and the absorbance of the sample was measured before water replacement. The drug release experiments with water replacement were typically conducted at room temperature, but in some instances a higher temperature was used to investigate the effect of temperature on drug release rates.

In some experiments, the extraction step was eliminated and the drug release experiments described above were conducted immediately after polymerizing the gel.

2.2.5.2. Timolol release in DI water without water replacement These experiments were performed to determine the time required for equilibrium to be established during the process of drug release from the microemulsion-laden gels. The protocols for these experiments were identical to those described above, except that the gel was kept in the same 3 ml DI water during the entire course of the drug release experiments. These experiments were conducted only for gels loaded with me A, and only at room temperature.

2.2.5.3. Timolol release from Pluronic microemulsion-laden gels in PBS Since DI water may not be a good mimic of tears, it was decided to perform timolol release experiments from Pluronic microemulsion-laden gels in phosphate buffered saline (PBS). Protocols identical to those described above were followed, except that the DI water was replaced by PBS or saline in the extraction and the drug release steps.

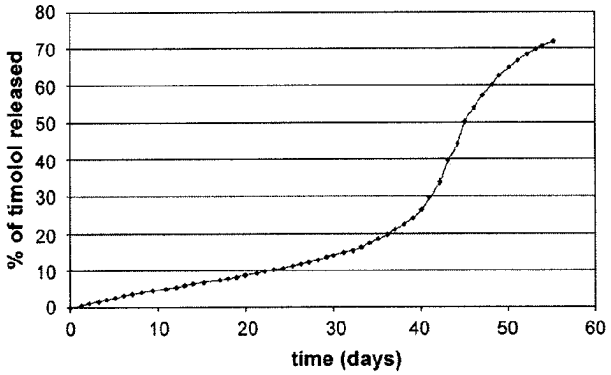

Fig. 1. Cumulative percentage release from the control PHEMA gels with water replacement every 24 h. The error bars denote standard deviation, $n = 2$.

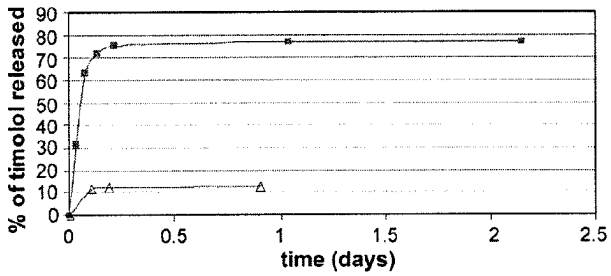

Fig. 2. Cumulative percentage release from the control gels into 3.5 ml PBS without PBS replacement (squares). After two days, PBS was replaced with fresh 3.5 ml PBS, and the cumulative release was measured (triangles).

3. Results and discussion

3.1. Drug release from HEMA gels loaded with timolol (control)

Control HEMA gels do not contain any microemulsion and these were loaded with drug by directly dissolving the timolol base in the polymerization mixture. Fig. 1 plots the cumulative percentage release from the control gels for the case of water replacement every 24 h. The data shows a very slow release with about 1% of the drug diffusing out each day. The release rates are almost linear for the first 40 days and then increase significantly in an almost exponential manner. Finally, the rates level off when a majority of the drug loaded in the gel is released. The cumulative drug release profiles are thus sigmoidal in shape. This behavior is interesting because typically in a diffusive system, the release rates decrease with time because of the reductions in concentration. The only plausible reason for the increase in the release rates after about 40 days is degradation of the gel. Fig. 2 plots the cumulative percentage release from the control gels in 3.5 ml PBS without any fluid replacement. After equilibrium was reached, PBS was replaced and the cumulative release for the second release is also plotted in Fig. 2. The results in Fig. 2 are in sharp contrast with those in Fig. 1 as the drug release into the PBS in very rapid, and it equilibrates in about 4 h. The cause of the large difference between the release behavior in DI water and in PBS can be explained by

the difference in partition coefficients of timolol base between the gel and DI water and between the gel and PBS. The partition coefficients for timolol maleate between HEMA hydrogel and DI water are concentration dependent, and the values in the concentration range of our experiments are much greater than the partition coefficient in PBS, which is relatively independent of concentration and has a value of about 5 [44]. The partition coefficients in DI water become larger than 100 at concentrations comparable to those in the release experiments described above and consequently a majority of the drug in the gel is bound to the polymer matrix and is not available to diffuse. This explains the slow release in DI water compared to PBS in which the partition coefficient is only about 5 and so a much larger amount of drug is available for diffusion. The significant differences in partition coefficients between PBS and DI water are due to the fact that in PBS almost the entire drug is expected to exist in the protonated form, which is highly soluble in water, but in DI water a fraction of the drug will exist as the base form, which has a limited solubility in water.

3.2. Drug release experiments for Pluronic microemulsion-laden gels

3.2.1. Timolol release in DI water with water replacement every 24 h

Fig. 3 plots the cumulative percentage release during the drug release experiments from a gel loaded with meA as a function of time. The results show that the gel releases drug for about 25 days, during which about 55% of the drug has diffused out. The type meA gel (gel loaded with meA) loses about 17.5% of the drug in the extraction step. Thus, the total drug release amounts to about 75% of the drug loading. A fraction of the loaded drug may be irreversibly trapped in the gel, and additionally, a fraction of the drug may be lost in the process of extracting the timolol base into the oil phase. The cumulative drug release profiles are sigmoidal for this case also. Furthermore, the release rates from these microemulsion-laden systems are faster than those from the HEMA control. Both of these interesting observations could be explained by considering the effect of ethyl butyrate which is the oil phase of the microemulsion. Since the oil has a finite solubility in the HEMA matrix, it may also diffuse, and this diffusion could lead to enhanced drug transport due to the high solubility of the drug in oil. Also, the presence of oil is likely to result in a weaker gel, which could cause faster transport and degradation.

In order to test the hypothesis that oil transport enhances drug transport, it was decided to measure drug transport at elevated temperatures at which both the oil and the surfactant have higher solubilities in water and in HEMA. Fig. 3 also shows the effect of an increase in the extraction temperature on the subsequent drug release which was conducted at room temperature. It was speculated that an increase in the extraction temperature will result in a larger loss of oil and surfactant in the extraction step, and that should lead to a faster drug release in the subsequent drug release experiments. The data in Fig. 3 is in agreement with this speculation. The release rates are faster for the gels which were exposed to a higher extraction temperature.

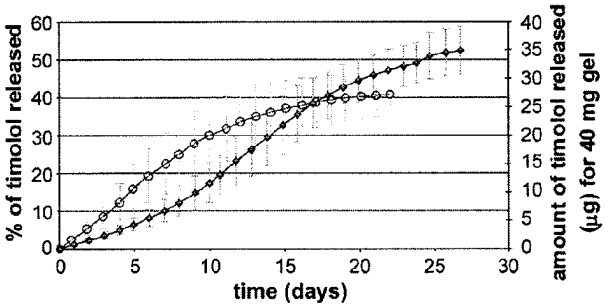

Fig. 3. Timolol released from meA-laden gel in DI water without water replacement. Solid diamonds represent experiments done with extraction at room temperature ($n = 4$). Hollow circles represent experiments done with extraction at 60 °C ($n = 2$).

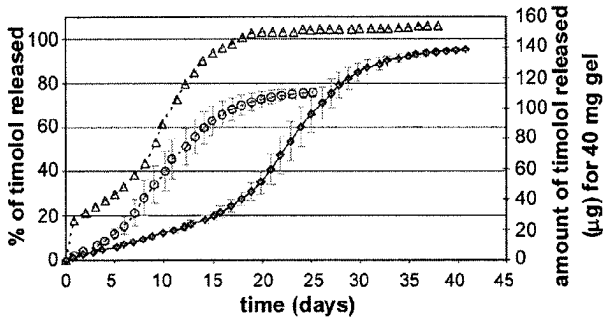

Fig. 4. Timolol released from meD-laden gel in DI water without water replacement. Solid diamonds represent experiments done with extraction at room temperature ($n = 2$). Hollow circles represent experiments done with extraction at 60 °C ($n = 2$). Hollow triangles represent release experiments done at 50 °C.

To further understand the contributions from oil transport to the drug transport, it was decided to entrap microemulsions with different oil and drug loadings. Fig. 4 plots the cumulative drug release profiles for gels loaded with microemulsion D, which have similar amount of oil (ethyl butyrate + timolol base) as microemulsion A, but have a higher ratio of drug to ethyl butyrate. Based on the above hypothesis, it could be speculated that a reduction in ethyl butyrate loading will lead to slower transport. The gels loaded with meD release drug for about 35 days, which is longer than the release time for meA gels. The effect of an increase in extraction temperature for meD gels is similar to that for meA gels. These gels release for about 20 days which is longer than the meA gels exposed to the higher extraction temperature. For meD gels, additional drug release experiments were conducted at elevated temperatures with extraction at room temperature. This data is also shown in Fig. 4. An increase in temperature during the release experiments leads to faster release without changing the sigmoidal shape of the curve. The measured cumulative release in this case exceeds 100%, which is due to errors in measurements. Fig. 5 plots the cumulative drug release profiles for gels loaded with microemulsion B which have the same ratio of drug to oil as meA but a higher microemulsion loading in the gel. Since the oil to drug ratio for meA and meB are similar, their

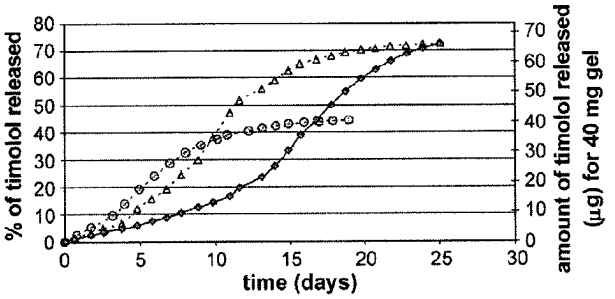

Fig. 5. Timolol released from meB-laden gel in DI water without water replacement. Solid diamonds represent release experiment done at room temperature ($n = 2$). Hollow triangles represent release experiment done at 50 °C. Hollow circles represent experiments done with extraction at 60 °C ($n = 2$).

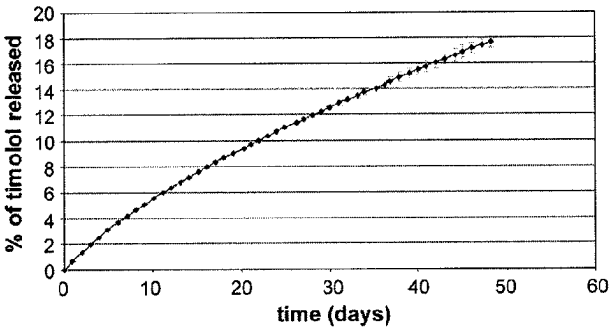

Fig. 6. Timolol release in DI water for meC-laden PHEMA gels. The error bars denote standard deviation, $n = 2$.

drug release profiles are expected to also be similar, which is indeed the case.

To further understand the role of oil in drug transport and also to determine whether the surfactants provide any barrier to transport it was decided to entrap microemulsions of timolol base (meC) in the gels. Since these systems have no ethyl butyrate, the drug transport is expected to be slower than that for meA and meD gels, and furthermore if the surfactants do not provide any barrier to transport, the release rates from these systems are expected to be similar to those from the control p-HEMA gels. The release experiments for gel C were stopped after about 50 days during which only about 20% of the drug diffused out (Fig. 6). In the first 40 days, the drug release profiles from gels loaded with meC are similar to those from the control gels with no surfactant, and this suggests that the surfactant covered interface does not retard transport.

3.2.2. *Timolol release in DI water without water replacement*

These experiments were performed to determine the equilibrium release time for the microemulsion-laden gels. These equilibrium experiments were only done on gel A. These gels lost 17.5% of the entrapped drug during the extraction phase. The results of drug release experiments (Fig. 7) show that about 8% of the entrapped drug diffuses out in a period of about 10 days. Based on this data the partition coefficient of timolol in gel A is about 800.

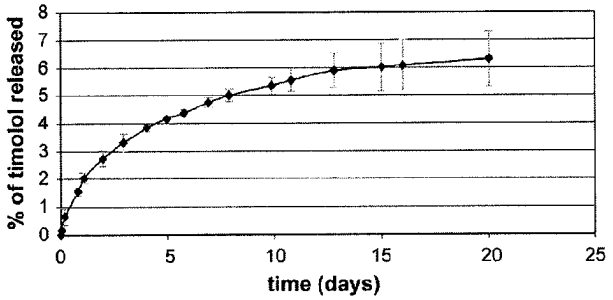

Fig. 7. Timolol released in DI water without water replacement for meA-laden PHEMA gels. The error bars denote standard deviation ($n = 4$).

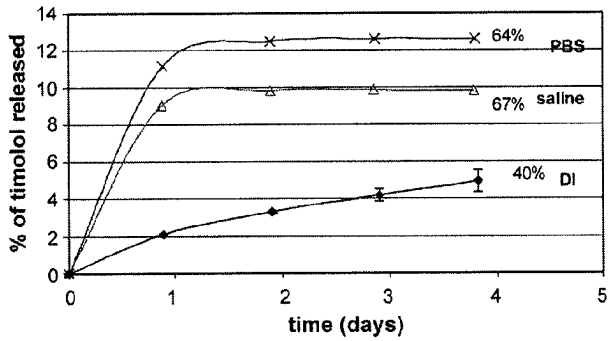

Fig. 8. Release of timolol from meA-laden gels into DI water ($n = 2$), PBS, and saline. The numbers on the curves represent the percentage of timolol loss during 5 h extraction in mediums identical to the release mediums which are the DI water, PBS, and saline. Solutions were changed every 24 h in the drug release experiments.

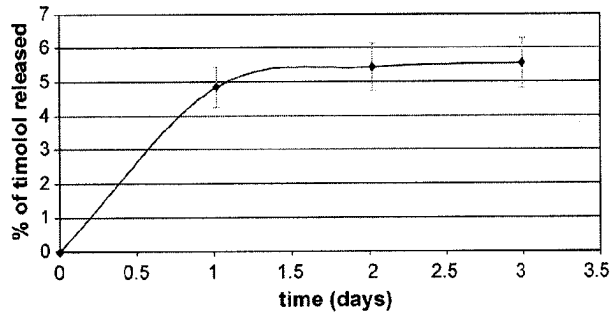

Fig. 9. Drug release in saline for gel D. Gels were first soaked in 10 ml saline for 5 h followed by soaking in fresh 3 ml saline, which was replaced every 24 h. 89% of timolol was lost during extraction ($n = 2$).

3.2.3. *Timolol release from Pluronic microemulsion-laden gels in PBS*

To better mimic tears, it was decided to perform timolol release experiments from Pluronic microemulsion-laden gels in PBS. The release in saline and PBS was much more rapid compared to the release in DI water. The extraction phase for these studies was conducted in 10 ml of saline. In both PBS and saline, about 90% of the drug diffused out during the extraction phase, and the remaining amount is released in the first 1.5 h of the drug release experiments (Figs. 8 and 9). This result is expected because the surfactants do not retard transport

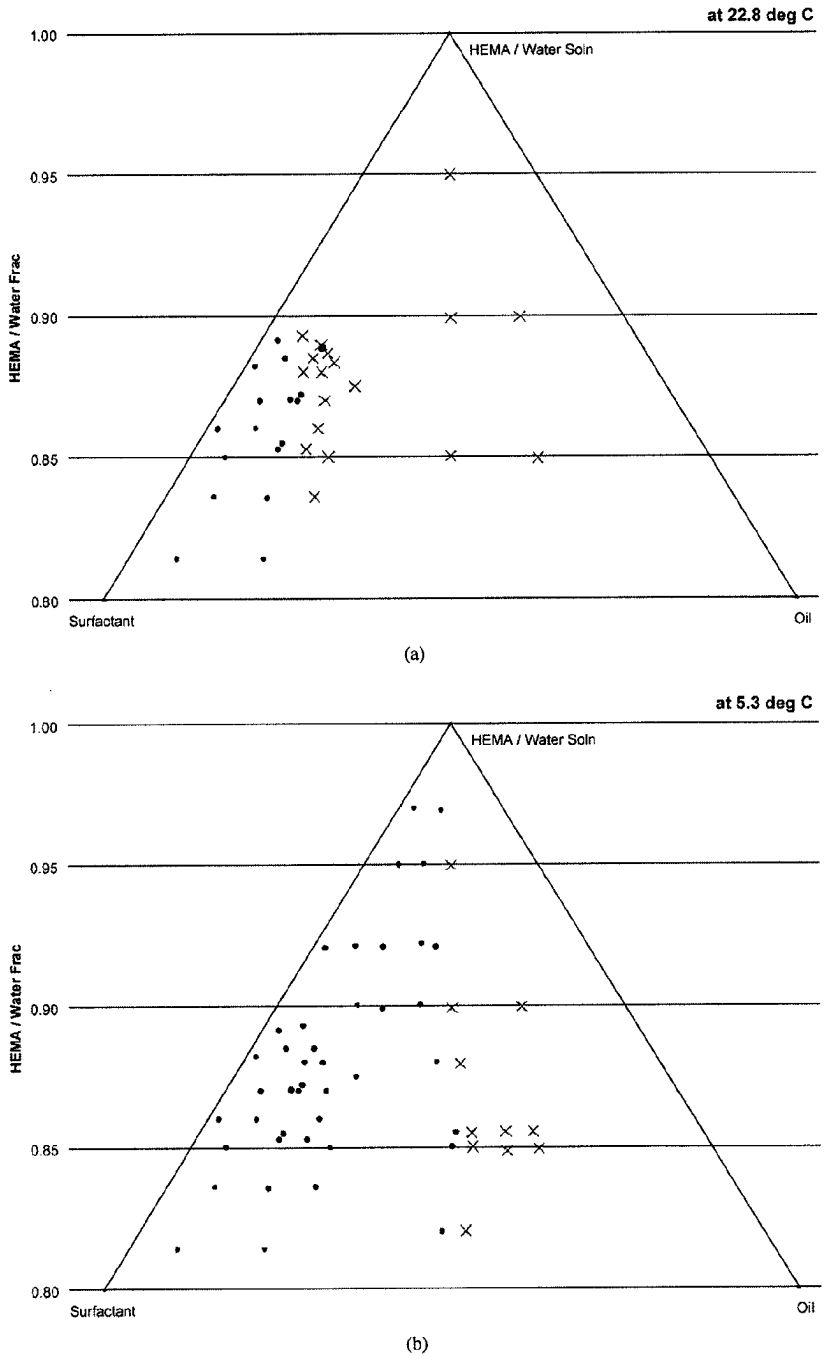

Fig. 10. Pseudo-phase diagrams for the six component microemulsion at (a) room temperature and (b) 5 °C. Circles represent transparent compositions and crosses represent hazy and opaque compositions.

and so the higher solubility of the drug in the PBS is expected to lead to a rapid release.

3.2.4. *Pluronic microemulsions with water–HEMA as the continuous phase*

It may be possible that the surfactant covered interface does not provide any barrier to transport because the microemulsions may be getting destabilized after HEMA addition to the microemulsion. To ensure that the microemulsions are stable after HEMA addition, it was decided to prepare microemulsion in HEMA–water solutions so that these can be polymerized without significant changes to composition. However, ethyl butyrate, which is the oil phase in the microemulsions, is highly soluble in a 40% water–60% HEMA mix, which is the compo-

Table 4
Effect of salt (a) and sodium hydroxide (b) concentrations on the solubility of ethyl butyrate in 60/40 HEMA/water solution. On adding 0.25 g NaCl and 3 ml of 2 N NaOH to 25 g of 60/40 HEMA/water solution, the oil solubility reduces to 1%. All solubility data is on w/w basis

(a)

Weight % of NaCl in 60/40 HEMA/water solution	Weight % of soluble ethyl butyrate in solution at 25 °C
0	10.1
0.5	6
1	3.3

(b)

Milliliters of 2 N NaOH in 25 g of 60/40 HEMA/water solution	Weight % of soluble ethyl butyrate in solution at 25 °C
0	10.1
0.5	8.8
1	8
2.5	6.5
3.25	2.8
4	1.4

sition of the solution that is polymerized to form the hydrogel. In order to minimize the solubility of the oil in the continuous phase, it was decided to increase the pH and the salt concentration of the continuous phase. The effect of salt and NaOH concentration on the solubility of ethyl butyrate in water–HEMA solutions are shown in Table 4. Based on this data, the composition of the continuous phase was eventually fixed to be HEMA/H_2O/NaCl/2 N NaOH solution = 53.6:35.8:1.6:9 on w/w basis. The solubility of ethyl butyrate in this mixture was determined to be 1%. Oil and surfactant were then added to this mixture in various ratios and the optical clarity of the system was noted. The compositions explored in these experiments at two different temperatures are indicated in the phase diagrams shown in Figs. 10a and 10b. In all of these figures, an '×' mark indicates phase separation, and an '•' marks a clear single phase. The transparent mixtures were also placed in between two polarized sheets but no change in polarization was detected, which eliminated the possibility of existence of liquid crystalline phases. The clear regions were thus inferred to be either single phase solutions or microemulsions. No effort was made to clearly mark the transition from single phase to microemulsions because the gels were prepared only with systems that had oil in excess over the solubility limit of 1% w/w.

3.3. Particle size distributions

The particle size distributions of some of the transparent compositions were measured to verify the existence of microemulsions. The distributions for three transparent compositions are shown in Fig. 11. The compositions of these three systems are given in Table 5. Each of these systems contains more oil than the solubility limit of 1% w/w. The mean particle sizes of the microemulsions depend on the ratio of surfactant to oil, and vary from 20 to 35 nm as the oil to surfactant ratio increases from 0.103 to 0.165. Also the distribution widens as the oil to surfactant ratio increases.

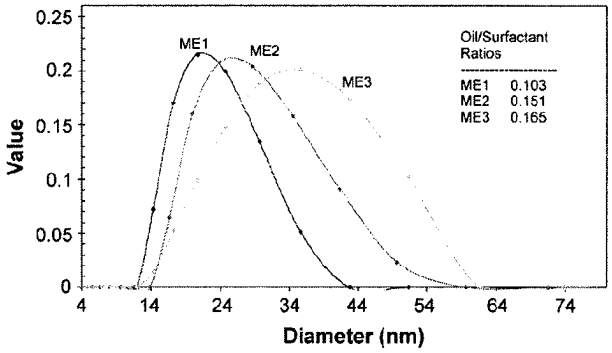

Fig. 11. Particle size distributions for the polymerizable microemulsions. The exact compositions of the three systems reported here are given in Table 5.

Table 5
Compositions of microemulsions utilized in light scattering measurements

	Pure component compositions (wt%)		
	ME 1	ME 2	ME 3
HEMA	44.295%	44.836%	45.903%
H_2O	29.530%	29.891%	30.602%
NaCl	0.738%	0.747%	0.765%
2 N NaOH	9.449%	9.565%	9.792%
Pluronic F127 surfactant	14.500%	13.004%	11.109%
Ethyl butyrate	1.487%	1.958%	1.829%

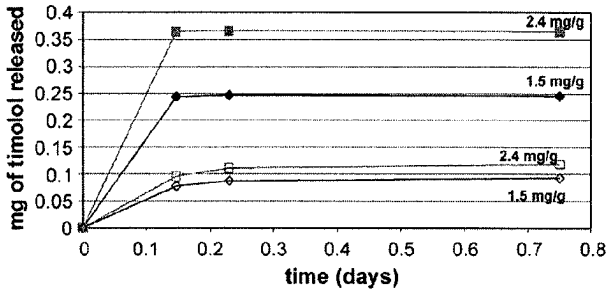

Fig. 12. Comparison of release of timolol in DI water (hollow markers) and in saline (solid markers) for stable Pluronic microemulsion in HEMA/water mixture. Release data was obtained at 3.5, 5.5, and 18 h after gels were put in 10 ml DI water or saline. Two loadings of timolol in gel were used for these experiments (1.5 and 2.4 mg timolol/g dry gel). No timolol release was observed after 3.5 h in saline but release continued in DI water.

3.4. Polymerization of the microemulsion

Timolol base was added to some of the transparent compositions, and the mixture was then polymerized to yield 200 μm thick gels. After polymerizing the microemulsions, drug release studies were conducted both in PBS and in DI water with protocols identical to those described above. These experiments showed that these systems also had a very rapid release in PBS and a slow release in DI water (Fig. 12).

3.5. Mechanism of drug transport

Following are the main features of the drug release from the PHEMA gels loaded with microemulsions:

(1) The release rates are sigmoidal for both the control and the microemulsion-laden gels.
(2) The release rates in PBS are much more rapid than in DI water both for the control gels and the microemulsion-laden gels.
(3) The release rates from gels loaded with microemulsions in DI water are larger than that for the control gels, and are correlated to the oil content of the gel.
(4) The release rates both during the initial extraction and the subsequent drug release experiments increase with increasing temperature.
(5) The release rates from the gels that were prepared by polymerizing microemulsions that contain HEMA are also much more rapid in PBS compared to that in DI water.

The sigmoidal shape of the release from the control suggests that the gel must be degrading over a period of a month. The same argument can explain the sigmoidal shape for the microemulsion-laden gels. The time at which the rates begin to significantly increase can be considered as the time at which the gel suffers significant degradation. This time is shorter for the microemulsion-laden gels because the presence of oil and microemulsions leads to formation of grain boundaries in the gel [45]. Furthermore, the dependence of transport rates on oil content could either be due to alteration of the gel microstructure due to oil, or due to coupling between the drug and the oil transport. The significant differences between transport in DI water and in PBS are due to differences in the partition coefficients. The partition coefficient is significantly larger in DI water which implies that a very large fraction of the drug in the gel is either bound to the polymer or is dissolved in the oil phase, and so is not available for diffusion. The dependence of transport rates on temperature are expected because of the increase in solubilities of the oil and the drug. The fact that the release rates are not slowed down by the microemulsion-laden gels shows that the surfactants at the interface of the microemulsions do not offer substantial transport resistance to timolol. It is also plausible that addition of HEMA to the microemulsions leads to destabilization, and solubilization of all the components, and consequently there is no slowdown of release. However, the microemulsions that contained a water–HEMA as the continuous phase are certainly stable as evident from light scattering measurements. The fact that the release rates from the gels prepared by polymerizing these is also very rapid eliminated the destruction of the microemulsions, or reduction in the packing at the interface as a potential reason for the lack of any barrier to transport. Thus, it seems logical to conclude that the Pluronic microemulsions do not impede the transport of timolol from inside the oil drops to the continuous phase, and that the transport is controlled by the gel. The only effect that the microemulsions have on the transport is through alteration of the gel structure [45], and partially due to coupling of the oil and the drug transport.

The rapid release of timolol from these systems in PBS makes them unsuitable for contact lens applications. However, it is very encouraging that these systems have a very large timolol loading, and thus these may find some applications in other areas such as transdermal patches for delivery of timolol or other drugs. These systems may also be useful for loading hydrophobic molecules that have a low solubility both in HEMA and in PBS. Furthermore, these systems may offer resistance to transport of larger molecules and so the Pluronic microemulsion-laden gels could be useful for controlled release of hydrophobic and larger molecules.

4. Summary

This paper focuses on measuring timolol transport from Pluronic microemulsions trapped in pHEMA gels. These gels could be useful for drug delivery applications because of large drug loading. To fabricate the microemulsion-laden gels, ethyl butyrate/water microemulsions stabilized by Pluronic F127 surfactant were prepared, and these were then added to HEMA followed by polymerization. It was speculated that addition of HEMA to the microemulsion may lead to destabilization and so we also synthesized six component microemulsions stabilized by Pluronic F127 that had ethyl butyrate as the oil phase and a solution of NaCl and NaOH in HEMA and water as the continuous phase. These microemulsions were polymerized to yield hydrogels. Both of these systems yielded transparent hydrogels with mechanical properties similar to those for HEMA gels. It was possible to obtain very large loading of timolol in these systems by dissolving the base form of timolol in the oil phase. Gels that had timolol loaded microemulsions exhibited a slow and extended drug release in DI water. In DI water, the transport of drug was faster in microemulsion-laden gels compared to control due to coupling between the oil (ethyl butyrate) and drug (timolol) transport. The transport rates showed a sigmoidal shape which was attributed to long term degradation of the gel. The transport rates for gels loaded with timolol microemulsions were faster in comparison to the control suggesting that the surfactant does nor retard drug transport in DI water. All of the gels exhibited a very rapid release in PBS and in saline due to higher solubility of timolol in these solutions compared to that in DI water. Thus, these systems are of limited utility for ophthalmic drug delivery. However, it is encouraging that these systems can have a very high timolol loading, and so these may find applications in other areas such as transdermal drug delivery, and also for delivery of hydrophobic molecules to eyes.

Acknowledgment

This research was partly funded by support from the National Science Foundation (CTS 0426327).

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